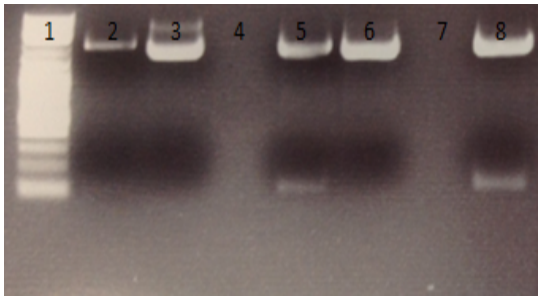
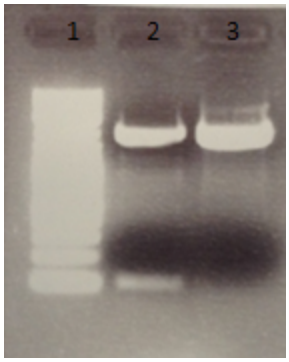
Day 21 - Monday - 06/30/14

- Alex and Chloe digested the BclA sequence with E and S and the CAEV sequence with X and P.
- Corbyn and Shawna performed a test digest of the 5 BCLA miniprepreps using E and P which was subsequently run on 2 gels. On the the first gel, samples 1&2 and 3&4 were confused, so we loaded the gel with samples 1&2 next to each other, 3&4 next to each other, and then 5. The first gel (1.66% agarose) can be seen below and was run at 70V for about 20 minutes. This gel showed that sample 5 had the BCLA insert as well as either sample 3 or 4. In order to see if either 3 or 4 had the correct BCLA insert, we ran another 1.46% gel with only ladder, BCLA sample 3 and 4 at 70V for about 20 minutes. This gel showed that the third miniprep of BCLA in biobrick format contained the correct size fragment, and can also be seen below.



Lane	Contents
1	Ladder
2	BCLAE-P 1 or 2
3	BCLA E-P 1 or 2
4	empty
5	BCLA E-P 3 or 4
6	BCLA E-P 3 or 4
7	empty
8	BCLA E-P 5



Lane	Contents
1	Ladder
2	BCLA E-P 3
3	BCLA E-P 4

Then, ligations were performed using the constitutive promoter, RBS, and kanamycin backbone (RP). These were then transformed and plated to incubate overnight at 37 degrees. Further, we prepared 5 liquid cultures from the CAEV plate that had grown over the weekend. The CAEV plate was found to have about 35 colonies total, and a picture of the plate can be found in the plate pictures folder in this week.

- Kayla and Mike test digested all five 22A minipreps and ran a gel to see if the arsenic promoter/RBS/ATF1/DT insert was present in the pSB1A3 plasmid backbone. Minipreps 1, 3, 4, and 5 only had a band around 4 kb, which is the size of the plasmid and the insert combined. In the lane for miniprep 2, the band for the full plasmid and a smaller one for

the insert were present. Because miniprep 2 appeared to have the plasmid with the insert, a three new liquid cultures were made from the glycerol stock of the colony used to make miniprep 2 and the plasmid was prepared for sequencing.

Day 22 - Tuesday- 07/01/14

- Alex and Chloe prepared Ligations using the following volumes:

	BC1	BC	BC2
pSB1A3 Volume (uL)	2	2	2
BclA Volume (uL)	2	2	1
CAEV Volume (uL)	1	2	2
dH2O Volume (uL)	12	11	12
Ligase Buffer Volume (uL)	2	2	2
Ligase Volume (uL)	1	1	1
Total Volume (uL)	20	20	20

A second ligation was prepared with BclA and YFP.

	BY1	BY	BY2
pSB1A3 Volume (uL)	2	2	2
BclA Volume (uL)	2	2	1
YFP Volume (uL)	1	2	2
dH2O Volume (uL)	12	11	12
Ligase Buffer Volume (uL)	2	2	2
Ligase Volume (uL)	1	1	1
Total Volume (uL)	20	20	20

An agglutination assay test was set up with 1:2 diluted OD_{595} of 1.3 5A culture (additionally diluted 1:2 in wells) with the following dilutions of antibody:

1:50	1:100	1:200	1:400	1:800	1:1600	1:3200	1:6400	1:12800	1:25600	1:51200

Liquid cultures for the interlab study were prepared

- Kayla and Mike received the results of the plasmid sequencing. Unfortunately, both the forward and reverse sequences were much shorter than the plasmid sequence and did not align with the sequence of the insert. However, because the insert band appeared on the gel the previous day, the banana odor construct testing was started. Five 5 mL liquid cultures, diluted 1:5 from the overnight cultures, were prepared and placed in the shaker for 2 hours. After 2 hours, 2.8 uL of isoamyl alcohol were added to each tube along with varying amounts of a 1:10 dilution of 0.5M arsenite. The contents of each tube can be seen in the table below. Once the arsenite and isoamyl alcohol were added, tube were placed in the shake for an additional 2 hours. Then, tubes were checked for banana odor because the bacteria containing a plasmid with a proper insert should be able to convert isoamyl alcohol to isoamyl acetate in the presence of arsenic. Because the tubes did not smell like banana after two hours, they were left in the shaker overnight. Digests of all five of the 22A minipreps were left in a 37^{o} water bath overnight.

  Banana Odor Generator Tubes

Tube	1:5 Culture Volume (mL)	Isoamyl Alcohol Volume (uL)	Isoamyl Alcohol Concentration	1:10 Arsenite Volume (uL)	Arsenite Concentration
1	5	2.8	5mM	0	0 uM
2	5	2.8	5mM	1	10 uM
3	5	2.8	5mM	2.5	25 uM
4	5	2.8	5mM	5	50 uM
5	5	2.8	5mM	10	100 uM

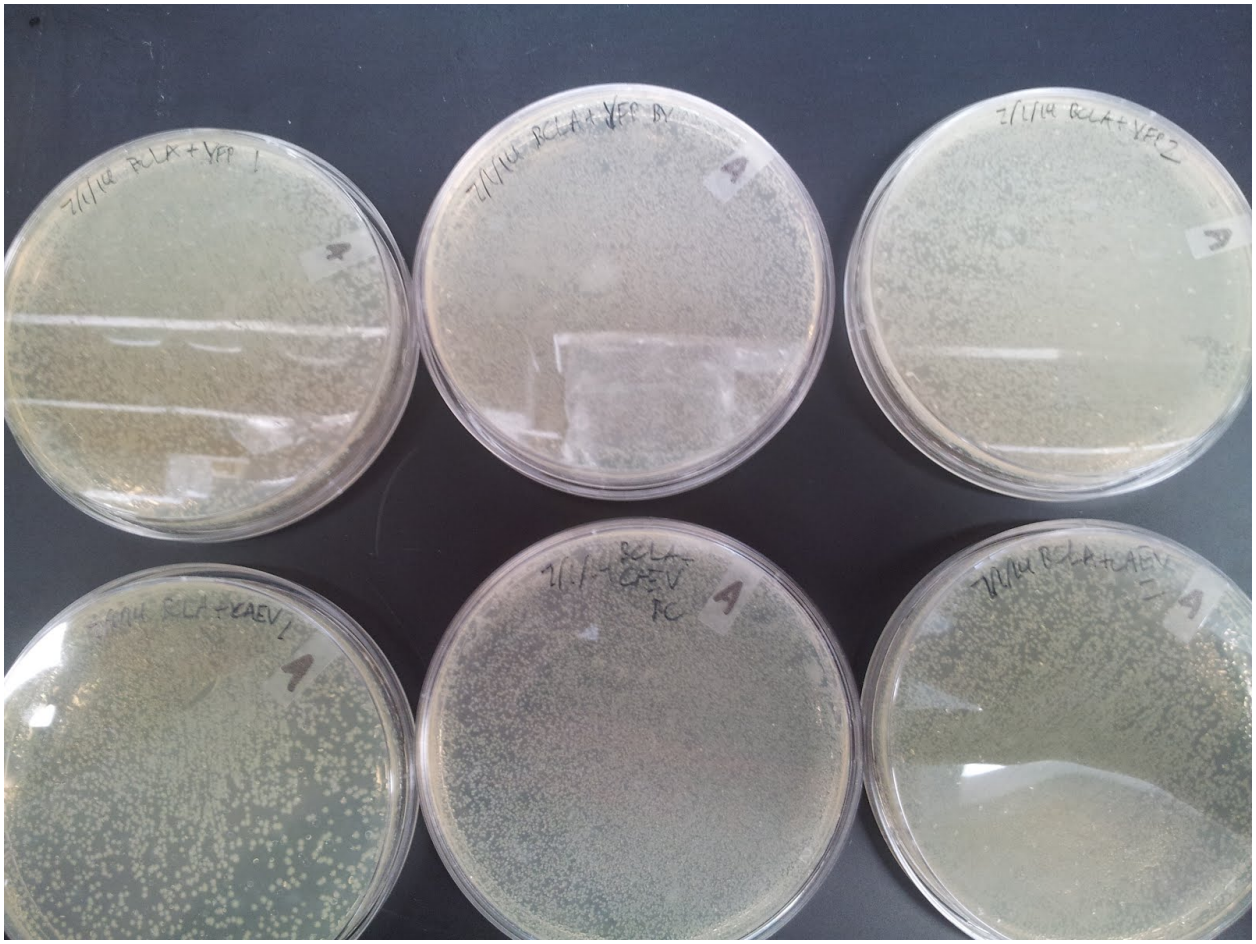
- Shawna and Corbyn collected the promoter+RBS plate from the warm room only to find that no colonies had grown. They then collected the 5 CAEV liquid cultures that had been placed in the 37 degree shaker overnight. A miniprep was prepared from each liquid culture, but once we tested the DNA concentration using the nanodrop, we found that all five samples had little to no DNA. This could potentially be an error in performing the miniprep protocol or possibly a problem with the samples themselves. We once again took 5 colonies from the CAEV biobrick plate and created a liquid culture that sat in the 37 degree shaker overnight, so we could try the miniprerps again tomorrow. Also, we performed another lligation of promoter and RBS into the Kanamycin backbone, using the

following volumes (see table below). The ligations were then transformed, plated, and left overnight in the warm room.

Sample	A	B	C	D	E
RBS Volume (uL)	.5	1	2	.5	1
Promoter Volume (uL)	1	1	1	2	2

Day 23 - Wednesday - 07/02/14

- Alex and Chloe prepared another 96-well plate with all 3 interlab study cultures (18C+19C, 19C+20C, and 17K)and ran it through the plate reader. Each culture was diluted in a set of 1, 1:2, and 1:10 and ran alongside a blank of LB. These results and all prior results have been posted in their own folder on the main page. Liquid cultures of BclA+YFP and BclA+CAEV ligations were prepared for miniprepping tomorrow. A photo of the plates was taken:



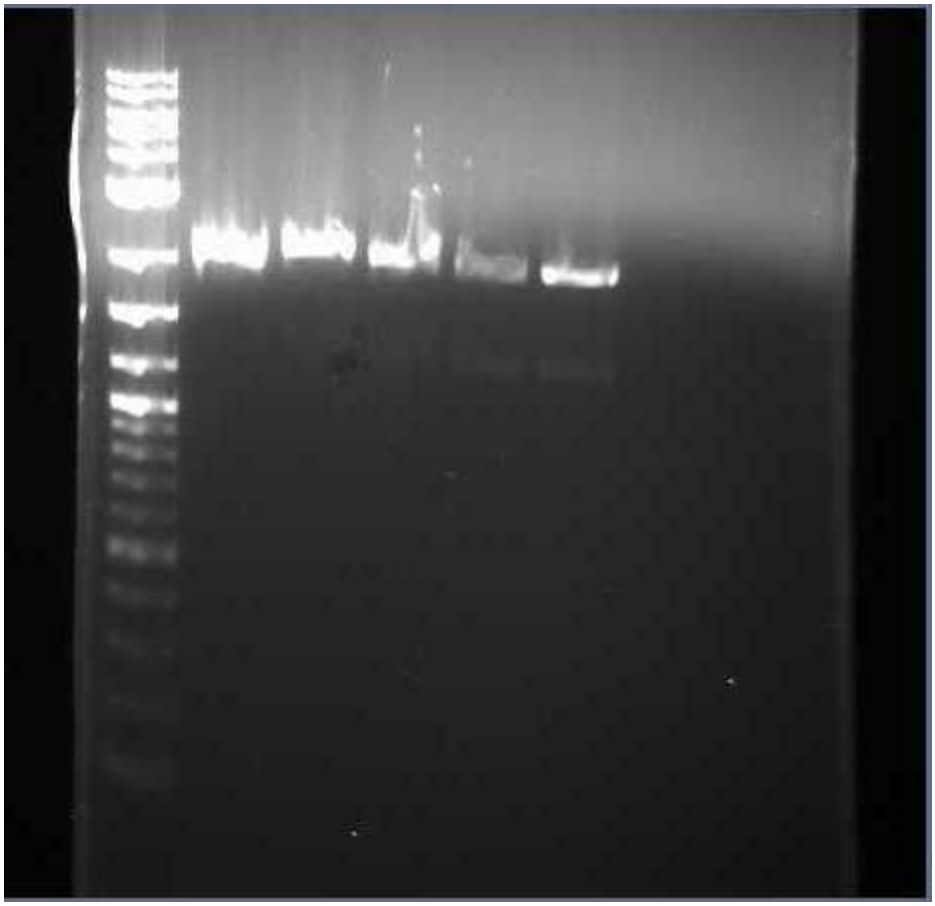
- Kayla and Mike checked the banana odor generator tubes that were left overnight. The tubes seemed to smell more like banana as the arsenite concentration increased, but

conclusions could not be drawn without running a gas spec. In the meantime, Mike prepared new liquid cultures to test varying isoamyl alcohol concentrations at a constant arsenite concentration. A list of tube contents can be found in the table below. After the cultures were allowed to grow in the 37^{O} shaker for a few hours, the appropriate volumes of isoamyl alcohol and 1:10 arsenite were added. They were then placed back in the shaker. In addition to the banana odor generator testing, the five miniprep digests that had been left in the water bath overnight were run on a gel at 82V for 1 hour. The bands were hard to see on the first gel, so a second one was run. The picture of the second gel and a table of the contents of each lane can be seen below. The lanes for minipreps 2-5 each had a faint band around 2 kb, indicating that the banana odor construct was correctly inserted into the plasmid.

Banana Odor Generator Tube Contents-Part 2

Tube	Culture Volume (mL)	Isoamyl Alcohol Volume (uL)	Isoamyl Alcohol Concentration	1:10 Arsenite Volume (uL)	Arsenite Concentration
1	5	0	0mM	2.5	25 uM
2	5	0.56	1mM	2.5	25 uM
3	5	2.8	5mM	2.5	25 uM
4	5	5.6	10mM	2.5	25 uM
5	5	14	25mM	2.5	25 uM

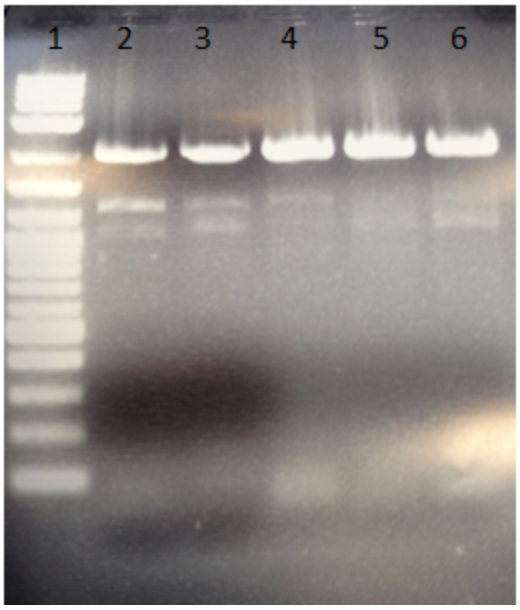
7/2/14 22A Miniprep Gel



Lane	Sample
1	Ladder
2	22A Miniprep 1 Digest
3	22A Miniprep 2 Digest
4	22A Miniprep 3 Digest
5	22A Miniprep 4 Digest
6	22A Miniprep 5 Digest

- Shawna and Corbyn once again collected the 5 Promoter+RBS transformation plates from the warm room and found no growth. We believe that this is occurring because we are not using the RBS that we created from oligos. We also collected liquid cultures of the CAEV biobrick, miniprepped all 5 of them, and found their DNA concentrations using the nanodrop. Unlike yesterday, the miniprep procedure was successful, and our DNA concentrations were good. We then perfomed a test digest (E-P) on the five biobricks and ran the samples on a 1.5% agarose gel at 70V for 40 minutes. A picture of the

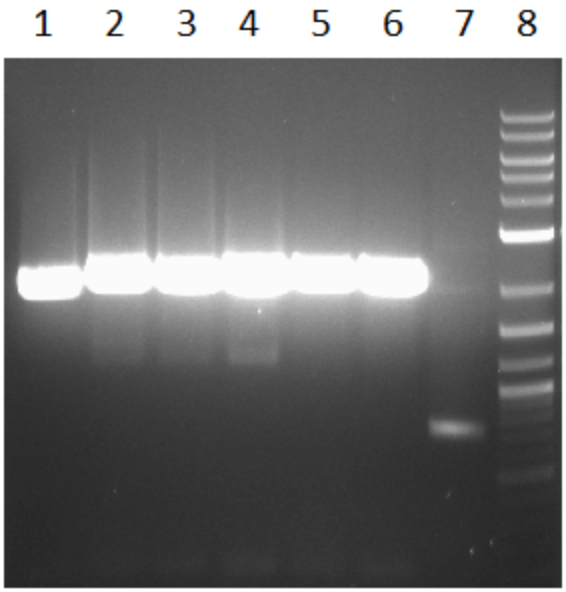
resulting gel can be seen below, with labeled lanes. As you can see, it looks as if once again only the plasmid band is present.



Lane	Contents
1	Ladder
2	CAEV Biobrick 1
3	CAEV Biobrick 2
4	CAEV Biobrick 3
5	CAEV Biobrick 4
6	CAEV Biobrick 5

Day 24 - Thursday- 07/03/14

- Alex and Chloe miniprepped the 6 liquid cultures for BclA+YFP and BclA+CAEV, digested them with E and P, and ran in the following gel:



Lane	Contents
1	BclA+CAEV (BC1)
2	BclA+CAEV (BC)
3	BclA+CAEV (BC2)
4	BclA+YFP (BY1)
5	BclA+YFP (BY)
6	BclA+YFP (BY2)
7	YFP PCR Product
8	Ladder

- Mike went ran a PCR for both Kanomycin and Chlor, which were later purified by Prof. Farny. The tubes are located in the Enzymes bTubeox.

- Mike also performed an experiment similar to the day before, but with varying amounts of isoamyl alcohol and a set concentration of arsenite. The results can be seen below

Tube	**Isoamyl alcohol (mM)**	**Odor test**
A	0	Like purely E. Coli, though it seems noticeably less strongly odorous
B	1	Less offensive than A and F, I smell a SLIGHT hint of sweet for a moment.
C	5	Doesn't last long, but there is a noticable fruity odor
D	10	not much different than C, a small "Blast: of mild banana odor
E	25	An intense banana odor for a few moments
F(no arsenite)	0	smells purely like E. Coli, as expected

As later found out by sequence analysis, our plasmid apparently did not contain the “bananarse” gene. We plan on performing an experiment to see if the cultures sans Arsenite will smell too similar to banana.